



Korea Advanced Institute of Science and Technology

<110> CONSTRUCTION OF NOVEL STRAINS CONTAINING MINIMIZING
GENOME BY Tn5-COUPLED Cre/loxP EXCISION SYSTEM

<130> 02730.0020.PCUS00

<140> 10/505,328

<150> PCT/KR02/02033

<151> 2002-10-31

<150> KR 10-2002-0009647

<151> 2002-02-22

<160> 13

<170> KopatentIn 1.71

<210> 1

<211> 2437

<212> DNA

<213> Artificial Sequence

<220>

<223> TnKGloxP

<400> 1

attcaggctg cgcaactgtt gggaagggcg atcgggtgcgg gcctcttcgc tattacgcca	60
gctgtctctt atacacatct caaccatcat cgatgaattc gagctcggta cccgggttga	120
actgcggatc ttgcggccgc aaaaattaaa aatgaagttt tgacgggtatc gaacccaga	180
gtcccgtca gaagaactcg tcaagaaggc gatagaagc gatgcgctgc gaatcgggag	240
cggcgatacc gtaaagcacg aggaagcggc cagccattc gccgccaagc tcttcagcaa	300
tatcacgggt agccaacgct atgtcctgat agcggtcgc cacaccagc cggccacagt	360
cgatgaatcc agaaaagcgg ccattttcca ccatgatatt cggcaagcag gcatcgccat	420
gggtcacgac gagatcctcg ccgtcgggca tccgcgcctt gagcctggcg aacagttcgg	480
ctggcgcgag cccctgatgc tcttcgtcca gatcatcctg atcgacaaga ccggcttcca	540
tccgagtacg tgctcgctcg atgcgatgtt tcgcttggtg gtcgaatggg caggtagccg	600
gatcaagcgt atgcagccgc cgcattgcat cagccatgat ggatactttc tcggcaggag	660
caaggtgaga tgacaggaga tcttgccccg gcaattcgcc caatagcagc cagtcccttc	720
ccgcttcagt gacaacgtcg agcacagctg cgcaaggaac gcccgtcgtg gccagccagc	780
atagccgcgc tgctcgtct tggagttcat tcagggcacc ggacaggtcg gtcttgacaa	840
aaagaaccgg gcgcccctgc gctgacagcc ggaacacggc ggcatacagag cagccgattg	900
tctgttgtgc ccagtcatag ccgaatagcc tctccacca agcggccgga gaacctgcgt	960
gcaatccatc ttgttcaatc atgcgaaacg atcctcatcc tgtctcttga tccactagat	1020
tattgaagca tttatcaggg ttattgtctc atgagcggat acatatttga atgtatttag	1080
aaaaataaac aaataggggt tccgcgcaca tttccccgaa aagtgccacc tgcatacatg	1140
aattgatccg aagttcctat tctctagaaa gtataggaac ttcgaattgt cgacaagctt	1200

gatctggctt atcgaaatta atacgactca ctatagggag accggaattc attattttgta	1260
gagctcatcc atgccatgtg taatcccagc agcagttaca aactcaagaa ggaccatgtg	1320
gtcacgcttt tcgttgggat ctttcgaaaag ggcagattgt gtcgacaggt aatggttgtc	1380
tggtaaaagg acagggccat cgccaattgg agtattttgt tgataatggg ctgctagtgtg	1440
aacggatcca tcttcaatgt tgtggcgaat tttgaagtta gctttgattc cattcttttg	1500
tttgtctgcc gtgatgtata catttgtgtga gttatagtgt tactcgagtt tgtgtccgag	1560
aatgtttcca tcttctttta aatcaatacc ttttaactcg atacgattaa caagggatc	1620
accttcaaac ttgacttcag cagcgtctt gtagttcccg tcatctttga aagatatagt	1680
gcgttctgt acataacctt cgggcattgg actcttgaaa aagtcatgcc gtttcatatg	1740
atccggataa cgggaaaagc attgaacacc ataagagaaa gtagtgacaa gtgttgacca	1800
tggaacaggt agttttccag tagtgcaaat aaatttaagg gtaagttttc cgtatgttgc	1860
atcaccttca cctctccac tgacagaaaa tttgtgcca ttaacatcac catctaattc	1920
aacaagaatt gggacaactc cagtgaagag ttcttctcct ttactcattt tttctaccgg	1980
taccggggga tcctctagag tcgacctgca ggcattgcaag cttggcgtaa tcatggtcac	2040
agctgtttcc tgtgtgaaat tgttatccgc tcacaattcc acacaacata cgagccggaa	2100
gcataaagtg taaagcctgg ggtgcctaata gagtgagcta actcacatta attgcgttgc	2160
gctcactgcc cgctttccag tcgggaaatc caagggcgaa ttcgagctcg gtaccgggcc	2220
ccccctcgag ggacctata acttcgtata gcatacatta tacgaagtta tattaagggg	2280
tccggatcct ctagagtaga cctctagagt cgacctgcag gcatgcaagc ttcaggggtg	2340
agatgtgtat aagagacagc tgcattaatg aatcggccaa cgcgcgggga gaggcgggtt	2400
gcgtattggg cgtcttccg cttcctcgct cactgac	2437

<210> 2
 <211> 1511
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> TnCloxP

<400> 2	
attcaggctg cgcaactgtt gggaagggcg atcgggtgcg gcctcttcgc tattacgcca	60
gctgtctctt atacacatct caaccatcat cgatgaattc gagctcggtg ccgcaaaaat	120
taaaaatgaa gttttaaatc aatctaaagt atatatgagt aaacttggtc tgacagttac	180
caatgcttaa tcagtgaggc accaataact gccttaaaaa aattacgccc cgccctgcca	240
ctcatcgag tactgttgta attcattaag cattctgcgc acatggaagc catcacagac	300
ggcatgatga acctgaatcg ccagcggcat cagcaccttg tcgccttgcg tataatatatt	360
gcccatggtg aaaacggggg cgaagaagtt gtccatattg gccacgttta aatcaaaact	420
ggtgaaactc acccagggat tggctgagac gaaaaacata ttctcaataa accctttag	480
gaaataggcc aggttttcac cgtaacacgc cacatcttgc gaatatatgt gtagaaactg	540

ccggaaatcg tcgtggtatt cactccagag cgatgaaaac gtttcagttt gtcctatggaa	600
aacggtgtaa caagggtgaa cactatccca tatcaccagc tcaccgtctt tcattgccat	660
acggaatttc ggatgagcat tcatcaggcg ggcaagaatg tgaataaagg ccggataaaa	720
cttgtgctta tttttcttta cggctcttaa aaaggccgta atatccagct gaacggtctg	780
ggtataggta cattgagcaa ctgactgaaa tgcctcaaaa tgttctttac gatgccattg	840
ggatatatca acggtggtat atccagtcat tttttctctc atttttagctt ccttagctcc	900
tgaaaatctc gataactcaa aaaatacgcc cggtagtgat cttatttcat tatggtgaaa	960
ggtggaacct cttacgtgcc gatcaacgtc tcattttcgc caaaagttgg ccaggggctt	1020
cccggtatca acagggacac caggatttat ttattctgcg aagtgatctt ccgtcacagg	1080
tatttattcg gcgcaaagtg cgtcgggtga tgctgccaac ttactgattt agtgatgat	1140
ggtgtttttg aggtgctcca gtggcttctg tttctatcag catcgatgaa ttgatccgaa	1200
gttcctattc tctagaaagt ataggaactt cgaattgtcg acaagcttga tctggcttat	1260
cgaaattaat acgactcact atagggagac cggaattcga gctcgggtacc gggccccccc	1320
tcgagggacc taataacttc gtatagcata cattatacga agttatatta agatcctcta	1380
gagtcgacct gcaggcatgc aagcttcagg gttgagatgt gtataagaga cagctgcatt	1440
aatgaatcgg ccaacgcgcg gggagaggcg gtttgcgat tgggcgctct tccgcttctt	1500
cgctcactga c	1511

<210> 3
 <211> 19
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> OE sequence

<400> 3	
ctgtctctta tacacatct	19

<210> 4
 <211> 34
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> loxP site

<400> 4	
ataacttcgt atagcataca ttatacgaag ttat	34

<210> 5
 <211> 996
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> KmR gene

<400> 5
gcaaaaatta aaaatgaagt tttgacggta tcgaacccca gagtcccgt cagaagaact 60
cgtcaagaag gcgatagaag gcgatgcgct gcgaatcggg agcggcgata ccgtaaagca 120
cgaggaagcg gtcagcccat tcgccccaa gctcttcagc aatatcacgg gtagccaacg 180
ctatgtcctg atagcgggtcc gccacaccca gccggccaca gtcgatgaat ccagaaaagc 240
ggccattttc caccatgata ttcggcaagc aggcacgcc atgggtcacg acgagatcct 300
cgccgtcggg catccgcgc ttgagcctgg cgaacagttc ggctggcgcg agcccctgat 360
gctcttcgtc cagatcatcc tgatcgacaa gaccggcttc catccgagta cgtgctcgct 420
cgatgcgatg tttcgcttgg tggtcgaatg ggcaggtagc cggatcaagc gtatgcagcc 480
gccgcattgc atcagccatg atggatactt tctcggcagg agcaaggatga gatgacagga 540
gatcctgccc cggcacttcg cccaatagca gccagtcctt tcccgttca gtgacaacgt 600
cgagcacagc tgcgcaagga acgcccgtcg tggccagcca cgatagccgc gctgcctcgt 660
cttgaggttc attcagggca ccggacaggt cggctcttgac aaaaagaacc gggcgcccct 720
gcgctgacag ccggaacacg gcggcatcag agcagccgat tgtctgttgt gccagtcac 780
agccgaatag cctctccacc caagcggccg gagaacctgc gtgcaatcca tcttgttcaa 840
tcatgcgaaa cgatcctcat cctgtctctt gatccactag attattgaag catttatcag 900
ggttattgtc tcatgagcgg atacatattt gaatgtattt agaaaaataa acaaataggg 960
gttcgcgcga catttccccg aaaagtgcc cctgca 996

<210> 6
<211> 947
<212> DNA
<213> Artificial Sequence

<220>
<223> GFP gene

<400> 6
attatttgta gagtcatcc atgccatgtg taatcccagc agcagttaca aactcaagaa 60
ggaccatgtg gtcacgcttt tcggtgggat ctttcgaaag ggcagattgt gtcgacaggt 120
aatggttgtc tggtaaaagg acagggccat cgccaattgg agtattttgt tgataatggg 180
ctgctagtgt aacggatcca tcttcaatgt tgtggcgaat tttgaagtta gctttgattc 240
cattcttttg tttgtctgcc gtgatgtata cattgtgtga gttatagttg tactcgagtt 300
tgtgtccgag aatgtttcca tcttctttaa aatcaatacc ttttaactcg atacgattaa 360
caagggatc accttcaaac ttgacttcag cagcgtctt gtagttcccg tcatctttga 420
aagatatagt gcgttcctgt acataacctt cgggcattgg actcttgaaa aagtcatgcc 480
gtttcatatg atccggataa cgggaaaagc attgaacacc ataagagaaa gtagtgacaa 540
gtggtggcca tggaacaggt agttttccag tagtgcaaat aaatttaagg gtaagttttc 600
cgtatgttgc atcaccttca cctctccac tgacagaaaa tttgtgcca ttaacatcac 660
catctaattc aacaagaatt gggacaactc cagtgaaaag ttcttctcct ttactcattt 720

tttctaccgg taccggggga tcctctagag tcgacctgca ggcatgcaag cttggcgtaa	780
tcatggtcac agctgtttcc tgtgtgaaat tgttatccgc tcacaattcc acacaacata	840
cgagccggaa gcataaagtg taaagcctgg ggtgcctaata gagtgagcta actcacatta	900
attgcgttgc gctcaactgcc cgctttccag tcgggaaatc caagggc	947

<210> 7
 <211> 1069
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> CmR gene

<400> 7	
gcaaaaatta aaaatgaagt tttaaataca tctaaagtat atatgagtaa acttggtctg	60
acagttacca atgcttaatc agtgaggcac caataactgc cttaaaaaaa ttacgccccg	120
ccctgccact catcgagta ctgttgtaat tcattaagca ttctgccgac atggaagcca	180
tcacagacgg catgatgaac ctgaatcgcc agcggcatca gcacctgtc gccttgcgta	240
taatatttgc ccatggtgaa aacggggggcg aagaagttgt ccatattggc cacgtttaaa	300
tcaaaactgg tgaaactcac ccagggattg gctgagacga aaaacatatt ctcaataaac	360
cctttagggg aataggccag gttttcaccg taacacgcca catcttgcca atatatgtgt	420
agaaactgcc ggaaatcgtc gtggtattca ctccagagcg atgaaaacgt ttcagtttgc	480
tcatggaaaa cgggtgaaca aggggtgaaca ctatcccata tcaccagctc accgtctttc	540
attgccatac ggaatttcgg atgagcattc atcaggcggg caagaatgtg aataaaggcc	600
ggataaaaact tgtgcttatt tttctttacg gtcttttaaaa aggccgtaat atccagctga	660
acggctctgg tataggtaca ttgagcaact gactgaaatg cctcaaaatg ttctttacga	720
tgccattggg atatatcaac ggtggtatat ccagtgattt tttctccat tttagcttcc	780
ttagctcctg aaaatctcga taactcaaaa aatacgcccg gtagtgatct tatttcatta	840
tggtgaaagt tggaacctct tacgtgccga tcaacgtctc attttcgcca aaagttggcc	900
cagggtctcc cggatcaac agggacacca ggatttattt attctgcgaa gtgatcttcc	960
gtcacaggta tttattcggc gcaaagtgcg tcgggtgatg ctgccaaactt actgatttag	1020
tgtatgatgg tgtttttgag gtgctccagt ggcttctgtt tctatcagc	1069

<210> 8
 <211> 19
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> primer-pMOD<MCS>FP-1

<400> 8	
attcaggctg cgcaactgt	19

<210> 9
 <211> 22
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> primer-pMOD<MCS>RP-1

 <400> 9
 tcagtgagcg aggaagcgga ag 22

 <210> 10
 <211> 28
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> primer-Tn5Ext

 <400> 10
 agcatacatt atacgaagtt atattaag 28

 <210> 11
 <211> 35
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> primer-Arb1

 <400> 11
 ttgagcgata gacgtacgat nnnnnnnnnn gatat 35

 <210> 12
 <211> 20
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> primer-Arb2

 <400> 12
 ttgagcgata gacgtacgat 20

 <210> 13
 <211> 25
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> primer-Tn5Int

 <400> 13
 tcgacctgca ggcattgcaag cttca 25